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FINAL REPORT

APPLICATION OF NMR IMAGING TO IMPROVE STORAGE AND MALTING OF BARLEY

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Applications of NMR imaging to improve storage and malting of barley

Marco L.H. Gruwel[1] and Suzanne R. Abrams

National Research Council, Plant Biotechnology Institute
110 Gymnasium Place, Saskatoon, SK, S7N 0W9. [1])contact: marco.gruwel@nrc.ca

Abstract

Malting quality barley samples of the varieties Harrington, Manley and TR118, each from two locations in Saskatchewan, were collected directly from the producers and sent to China for storage. At regular intervals samples were shipped back to Canada for analysis consisting of germination studies, α-amylase tests, Magnetic Resonance Imaging (MRI) and Magnetic Relaxation (NMR) studies. Samples showing a decrease in germinative energy and elevated levels of α-amylase also showed a rapid uptake of water in the area between the embryo and the endosperm as observed by MRI. Using NMR relaxation experiments viable and non-viable barley samples could be distinguished after 2 hours of imbibition.

Technical Report

1. Introduction and Background

China imports ~1.5m tonnes (1998 data) of malting barley annually with Canada supplying 0.3-0.5 m tonnes, Australia ~0.8 m tonnes and the European Community 0.1-0.2m tonnes. Recently, some shipments of Canadian barley have lost viability in China when stored over the summer months so that China is now reluctant to take delivery of Canadian malting barley after April for storage and use during the summer. This has resulted in loss of potential sales of 100,000-200,000 tonnes of barley on an annual basis. As malting barley contributes significantly to the economy of Saskatchewan, a research team consisting of personnel from the NRC Plant Biotechnology Institute (Saskatoon, SK), Prairie Malt Ltd./Cargill Malt Americas (PML, Biggar, SK) and the Grain Research Laboratory of the Canadian Grain Commission (CGC, Winnipeg, MB) started research on this project.

Malting barley must exhibit high levels of germination (95% or higher) and be able to maintain its viability during transportation and during storage periods of up to one year. Loss of barley viability during storage is of concern to producers, marketers and end users of malting barley. It results in the down grading of malting barley to feed barley with an associated negative economic impact on producers.

In recent years, some shipments of malting barley to countries such as China have lost viability. These shipments were not always stored under ideal conditions. However, it was not clear if the loss of viability was caused by the storage conditions or by damage to the barley before shipment or a combination of the two.

The reasons for the loss of barley viability during storage are not clear, but it is often associated with low levels of sprouting or pre-germination. This can occur if the grain is exposed to excess moisture just before harvest or at some time between harvest and grain utilization. In general, maximum seed viability and vigour are reached after the final drying stage of seed maturation. From this point in time gradual deterioration occurs. Many factors such as genetics, pre-harvest effects, mechanical damage during harvest and handling, storage conditions (moisture, temperature) and microbial infections, influence the rate at which seeds lose viability. However, at present no clear picture of why seeds lose viability has emerged. This is partially due to different aging conditions, different species and different analytical techniques used by different researchers.

However, with the actual cause frequently unknown, the loss of seed viability is often attributed to the reduction of plasmalemma integrity. Using accelerated aging techniques on cucumber seeds, it has been shown that membrane deterioration, observed as a decrease in total phospholipid content, could be linked with a reduction in seed viability. The increasing loss of cell membrane functionality leads to an inability to maintain turgor and to osmotically respond to environmental changes, resulting in seed death. During water uptake plasmalemma degradation becomes more pronounced. Parallel to plasmalemma dysfunction, changes in cell compartmentalization indicate disruption to internal membranes. Changes in membrane integrity can be quantitatively characterized by EPR of water soluble spin probes which have to be added to the seeds.

Our main research objective was to identify specific parameters associated with a loss of barley viability during storage and handling in order to develop a test to predict loss of seed viability using simple MRI/NMR techniques and simultaneously investigate the water uptake pattern in barley kernels. One project objective, "To optimize parameters of the malting process" for barley with compromised viability, could not be pursued due to the significant reduction in barley viability (*vide infra*). Process development would economically not be feasible and such samples should be considered a loss. All other interim milestones, as defined in

the project application were successfully met. Details can be found in the separately submitted Progress Reports.

2. Experimental

2.1 Sample handling

Malting quality samples of Harrington barley (*Hordeum vulgare*, cv. Harrington), Manley (*Hordeum vulgare*, cv. Manley) and TR118 (*Hordeum vulgare*, cv. TR118), each from two locations in Saskatchewan, were collected directly from producers (1998 crop) by Prairie Malt Ltd./Cargill Malt Americas and sent to Nanjing, China in January 1999 following standard procedures for bulk shipments. Subsamples of these bulk samples were kept in Canada and stored at 5°C in plastic containers in December 1998. These are referred to as the control samples.

Sample shipments from China were received at intervals from May to October 1999. Samples were shipped from China in sealed plastic containers with screw caps and contained approximately 1 kg of material. The containers were completely filled to prevent kernel damage due to physical stress. Upon arrival in Canada, the samples were immediately stored at 5°C.

2.2 Kernels for NMR and MRI Measurements

For the purpose of relaxation time measurements (NMR), small batches of 15 kernels were imbibed at room temperature in distilled water using a 50 mL beaker, while shielded from air and light. At selected intervals a single kernel was removed and blotted with filter paper to remove any excess water attached to the husk. The blotted kernels were centered in a capped Zirconia MAS spinner (2cm length and 0.7 cm o.d.) and inserted into the NMR probe. For every NMR relaxation time experiment a new kernel was used and NMR relaxation time measurements were performed up to 4-5 times per imbibition time point used in the NMR experiments.

Micro imaging experiments were performed on samples taken from a germination dish. As only small samples of barley were available, Petri dishes (Fisher Scientific Ltd., Nepean, ON, Canada) with a diameter of 54 mm were used for 35 kernels in 1.5 mL water on Whatman #1 filter paper (Whatman Ltd., Maidstone U.K.). This method was adapted according to the EBC. Germination was performed in a dark cabinet at 20°C and as needed for MRI measurements, kernels were removed at regular intervals. After micro imaging, kernels were returned to a different Petri dish in order to follow germination progress with time. This would allow us to correlate MRI observations with the presence or absence of germination after visual inspection.

2.3 NMR Spectroscopy

All magnetic relaxation experiments were performed on a Bruker Avance DRX 360 Wide Bore machine, operating at 360.13 MHz for ^{1}H. Barley kernels were studied with a Bruker broad band Magic Angle Spinning (MAS) probe. With the exception of dry barley kernels, the samples were studied without spinning at 20°C. Relaxation studies on dry kernels used a sample spinning frequency of 2.5 kHz. The $\pi/2$ ^{1}H pulse length was 4.5 µs. Using a spectral width of 15.2 kHz and a recycle time of 4 s, 1H NMR spectra were acquired in 64 scans and stored in data blocks containing 4096 time domain points. Spin-lattice relaxation rates were measured with an inversion recovery sequence, π-τ-$\pi/2$-acq., with 14-16 different τ values. After Fourier transformation the signal amplitudes were fitted to a single exponential using a three parameter expression of the form, $M(t) = C[1 - B \exp(-R_1 t)]$. With C and B adjustable parameters, M(t) the signal amplitude at time t and R_1 the spin-lattice relaxation rate ($1/T_1$).

Studies of ^{13}C and ^{31}P were also performed on the broad band MAS probe. Each experiment used one barley kernel (single, whole seed analysis) which was inserted into a Zirconia spinner (2cm length and 0.7 cm o.d.). For ^{31}P experiments the sample was kept static but for the ^{13}C studies the kernels were spun at 2-5 kHz.

The ^{13}C and ^{31}P experiments discussed in this report all used Harrington barley with a high germinative power (1997 crop, 10.7% protein, 11.8% moisture, obtained from Dr. X.S. Yin, (PML). Please note that this sample was not shipped to China. Adamantane and KH_2PO_4 were used to optimize the acquisition parameters for the ^{13}C and ^{31}P MAS experiments, respectively.

2.4 MRI/Micro Imaging

MRI experiments were also performed on the Avance DRX 360, using a Bruker Micro-2.5 probehead and gradient system. All imaging experiments were performed at room temperature 20°C on single seeds mounted on a plexiglass foot and inserted in a 10 mm NMR tube. The relative humidity in the tube was maintained at 100% r.h., using a piece of wet cotton wool inserted in the cap of the NMR tube.

For the acquisition of micro images a Hahn spin-echo pulse sequence was used. Each echo experiment took 17 minutes, and used a Field of View (FOV) of 1.0 cm and a slice thickness of 0.6 mm. Each image consisted of a 256 x 256 data matrix, resulting in a pixel resolution of 39 µm. The Hahn spin echo pulse sequence consisted of 2 ms SINC3 pulses for selective excitation and refocusing with an echo time of 6.35 ms and a recycle delay of 1s.

2.5 α-Amylase Assay

α-Amylase assays were performed according to the method of MacGregor. The activity of the enzyme is expressed in iodine dextrin colour (IDC units). One IDC unit corresponds to the amount of enzyme required to lower the absorbance of a standard amylopectin β-limit dextrin digest

from 0.6 to 0.4 in 100 min. α-Amylase activity levels reported were the average of replicated analyses.

2.6 Germinative Energy

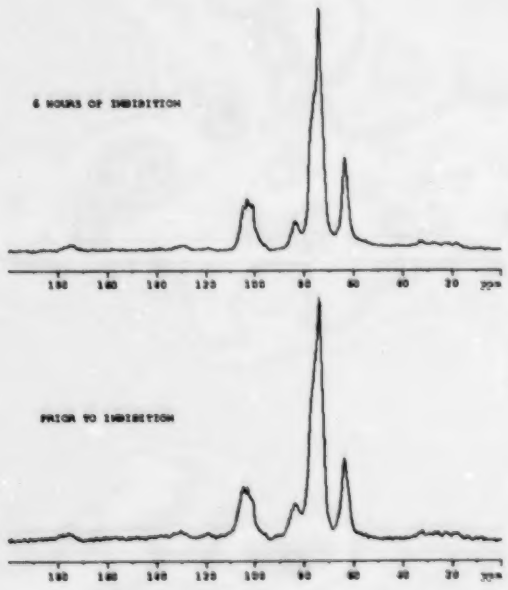
Barley kernels (100) were placed on two pieces of Whatman #1 filterpaper in a 9 cm Petri dish. Deionized water (4 mL) was added and the samples were kept in the dark at room temperature. Germinated kernels were counted at 24, 48 and 72 hrs., but only the 72 hr. values are reported here.

Replicates of 50 seeds from the sub-samples returned from China after 6 months storage were stained with a 10 $g \cdot kg^{-1}$ solution of Tetrazolium Chloride, according to the ISTA method. Kernels with non-staining embryos were classified as non-viable.

3. Results and Discussion

3.1. Multi Nuclear Spectroscopy; ^{13}C and ^{31}P NMR

Changes in seed metabolism can be followed by ^{13}C NMR of metabolic products. Many metabolites occur relatively immobilized in the kernel matrix. This results in very broad resonances when using conventional high resolution NMR. A special technique, Magic Angle Spinning (MAS), was applied in order to separate resonances of different metabolite groups (sugars, oils) in whole barley kernels. Additional signal enhancement and spectral resolution was obtained using cross polarization and high power ^{1}H decoupling (CPMAS).



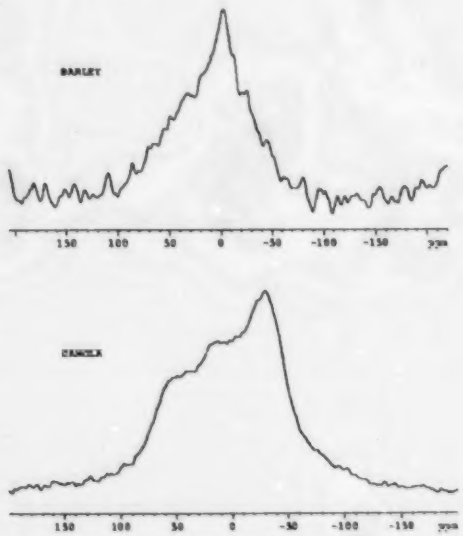


Figure 1. ^{13}C NMR spectra of Barley, prior to and after 6 hours of imbibition.

Figure 1 (bottom) shows a typical ^{13}C NMR spectrum of Harrington barley obtained with CPMAS prior to imbibition. The broad, and largest, resonance at 74 ppm results from the barley starch reservoir. Resonances at ~105 ppm and 64 ppm can also be assigned to the starch carbon backbone. The low amplitude resonances centred at 105 ppm are due to protein carbonyl groups, while methyl and methylene groups (mainly oils/fats) occur in the region of 10-40 ppm. Imbibition studies over a period up to 46 hours showed that no detectable changes occurred in the ^{13}C NMR spectrum. In Figure 1 (top), a typical ^{13}C CPMAS spectrum is shown after 6 hours of imbibition. Sugars and other substances produced by metabolic activity are expected to occur in 60-110 ppm region of the ^{13}C NMR spectra, however, in this spectral region starch resonances are dominant.

To eliminate the dominance of starch in the 60-110 ppm spectral region we studied the application of a special technique which allows the observation of non-protonated

Figure 2. Static ^{31}P NMR spectra of a barley and canola seed.

carbons while the resonances of protonated carbons can be made to vanish. This technique is based on the introduction of a delay in the signal acquisition. During this delay no ^{1}H decoupling occurs and signals from protonated carbon atoms rapidly dephase, leaving only those resonances which belong to non-protonated carbons. This technique was successful, however, the signal to noise ratio of the acquired spectra was not good enough to provide detailed information about new metabolites. Unfortunately, long-time signal averaging can not be performed due to the ongoing metabolism in the kernels during the experiments.

Small changes in the ^{13}C NMR spectra were observed to occur in the regions of 10-40 and 105 ppm. However, these changes were too small to resolve (signal averaging problem) within an appropriate time frame. It should be mentioned that after water uptake, the kernels become biologically active and their metabolic status changes continuously, excluding the possibility of long-time acquisitions (signal averaging) to obtain a spectrum with a better signal to noise ratio.

Using EPR it was recently shown that the loss of germinability with aging in wheat coincides with a considerably increased plasma membrane permeability of the embryo axis cells. In barley, a loss of germinability could thus also be related to changes in the phospholipid bilayer. An excellent tool to study the structure and dynamics of lipid bilayers is solid state ^{31}P NMR. The technique allows for the observation of possible membrane phase transitions and changes in local membrane dynamics. Figure 2 (top) shows a typical ^{31}P NMR spectrum of a static barley kernel. As for ^{13}C NMR, the signal to noise ratio remains low due to the limited time available for data acquisition. For comparison a ^{31}P spectrum of a canola seed is shown in Figure 2 (bottom), using exactly the same experimental conditions as for barley. The canola spectrum shows the typical powder pattern for intact lipid bilayers. A similar feature exists for the barley ^{31}P spectrum, however, due to the poor signal to noise ratio the features are not prominent.

3.2. Germination and α-Amylase results.

Final germination levels, α-amylase activity and Rapid Viscosity Analysis (RVA) data of barley samples received back from China are presented in the Tables 1,2,3 and 4 respectively. RVA data presents additional data provided by researchers from the CGC (*vide infra*). With the exception of the first control experiments which were performed in December 1998, all other test were performed in 1999 unless mentioned otherwise.

One sample each of Harrington, Manley and TR118 and the Schooner and Triumph varieties maintained high levels of germination throughout the storage period. Three samples, Harrington, Carrot River, SK; Manley, Tisdale, SK; and TR118 from Arborfield, SK, had lost a substantial amount of germinative energy by July. These samples continued to lose viability while the other samples showed stable levels of germinative energy through the last sampling date. Of the samples showing reduced viability, the TR118 sample from Arborfield, SK, was the worst. With the exception of TR118, Arborfield, SK, all control samples maintained their viability. Tetrazolium staining of the 6-months storage samples showed that the samples that were losing viability also contained significant numbers of dead embryos (caused by storage) while the aleurone still appeared to be active.

Cultivar	Germinative energy (%)					
		Storage Time (Months)				
(Origin)	Control	5	6	7	8	Control[a]
Harrington (St. Brieux)	97	100	100 100[b]	99	100	99
Harrington (Carrot River)	99	99	84 91[b]	72	63	96
Manley (Tisdale)	99	95	77 90[b]	59	51	98
Manley (St. Brieux)	100	99	100 100[b]	99	98	100
TR118 (Arborfield)	96	72	44 47[b]	23	20	87
TR118 (Nakorn)	99	99	100 100[b]	100	99	100
Schooner	-	100	95	96	91	99
Triumph	-	99	100	99	99	97

Table 1. Results of germinative energy studies of barley samples. [a])This control was performed in December 1999 while the first control was performed in December 1998.
[b])Percent viable embryos as determined by Tetrazolium chloride staining.

The samples with reduced germinative energy also displayed elevated levels of α-amylase. α-Amylase levels tended to increase during storage in China, but remained low in control samples. Increase in the level of α-amylase can thus be correlated with changes during the storage period. The TR118 from Arborfield showed highest α-amylase activity prior to shipment to China and levels tended to drift upwards during storage.

In addition to germination and α-amylase tests, the 5 and 6-months storage samples were also subjected to a Rapid Viscosity Analysis (RVA). This method provides a qualitative measure for the changes in rheological properties of seed reserves suspensions, *e.g.*, provide a measure for the α-amylase activity. The rapid viscosity analyzer is a recording viscometer that determines the pasting properties of starch in water/starch and water/ground grains suspensions. Only control samples and samples from two storage periods were studied with RVA. However, the results are significant to indicate the potential of the technique to predict excessive levels of α-amylase. Table 3 shows that all samples with reduced viability could be characterized by a time to peak viscosity smaller than 5 min. RVA measurements were in good agreement with α-amylase studies reported in Table 2. There appears to be a relationship between the maximum peak viscosity and the α-amylase level in the samples. The boundary between poor and good barley samples is not that well defined using maximum peak viscosity as a criterion, shown in Table 4. Levels less than 200-250 RVU (proportional to cP) should be considered suspect. On the other hand, the final viscosity

Cultivar	α-amylase (IDC units·g^{-1} D.M.)					
	Storage Time (Months)					
(Origin)	Control	5	6	7	8	Control*
Harrington (St. Brieux)	37	41	114	145	145	76
Harrington (Carrot River)	130	170	220	225	645	170
Manley (Tisdale)	365	231	375	343	388	304
Manley (St. Brieux)	75	59	114	120	165	93
TR118 (Arborfield)	643	1530	1620	1806	1660	348
TR118 (Naicom)	65	35	109	133	243	88
Schooner	-	37	82	110	81	47°
Triumph	-	31	69	103	122	63°

Table 2. *Results of α-amylase tests performed at CGC. Note: malt check contained 145,000 IDC units· g^{-1}. °)This control was performed in October 1999. *)Performed on the 5 month storage sample.*

Cultivar	Time to peak viscosity (min.)		
	Control	5 months storage	6 months storage
Harrington (St. Brieux)	5.57	5.60	5.60
Harrington (Carrot River)	4.92	5.02	5.02
Manley (Tisdale)	4.73	5.12	4.89
Manley (St. Brieux)	5.64	5.64	5.57
TR118 (Arborfield)	4.66	3.78	3.75
TR118 (Naicom)	5.80	5.60	5.80
Schooner	-	5.67	5.70
Triumph	-	5.74	5.70

Table 3. *Time to peak viscosity results of the Rapid Viscosity Analysis test. Data from CGC.*

(Table 4) appears to be very sensitive to the presence of α-amylase. Sound samples all show final viscosities in excess of 300 RVU while the poor samples showed values of 160 RVU or less.

Comparing the results listed in Tables 1 and 2 shows that samples showing poor viability (germination studies) appear to have elevated α-amylase levels. The results suggest that the observed loss of barley viability during storage in China is a result of pre- or post harvest sprouting. This hypothesis is supported by SEM studies showing degradation of the endosperm in TR118, Arborfield, SK, control samples. It should be noted that no specific variety-dependence was observed.

Cultivar	Peak viscosity (RVU)			Final viscosity (RVU)		
	Control	storage Month 5	storage Month 6	Control	storage Month 5	storage Month 6
Harrington (St. Brieux)	311.6	321.4	329.9	341.9	357.9	370.8
Harrington (Carrot River)	176.7	202.8	220.6	123.3	140.2	159.3
Manley (Tisdale)	120.4	145.2	152.4	55.4	96.6	93.7
Manley (St. Brieux)	257.6	286.2	275.5	299.6	316.2	312.5
TR118 (Arborfield)	91.7	60.6	64.0	37.2	10.0	9.6
TR118 (Naicom)	307.7	291.3	315.5	322.8	308.6	329.0
Schooner	-	243.1	267.8	-	306.1	340.3
Triumph	-	291.2	298.8	-	332.9	330.1

Table 4. *Peak and Final viscosity results of the Rapid Viscosity Analysis test. Data supplied by CGC.*

3. MRI studies of single kernels.

Micro imaging studies of barley kernels were performed using only one kernel per experiment. Barley samples with reduced viability (see Table 1) still contained viable kernels making the selection of individual kernels for micro imaging difficult. To maintain efficient data acquisition of non-viable kernels, batches with a known viability of approximately 50% or less were selected for imaging. According to this criterion the TR118 (Arborfield) samples stored for 6-8 months and the Manley (Tisdale) sample stored for 8 months were selected. Using the standard Petri-dish germination protocol, water uptake in barley kernels was followed as a function of time for selected samples. As it is not always clear whether or not a kernel germinates, especially during the early stages of germination, all kernels used for MRI were returned to a separate Petri-dish in order to follow germination after imaging. Using this control, MRI observations could directly be correlated with the germination history of the seed at a later stage.

Figure 3 shows a very simple model of a barley kernel in a reference frame. This frame will be used to explain the orientation of some of the images. The shaded rectangle

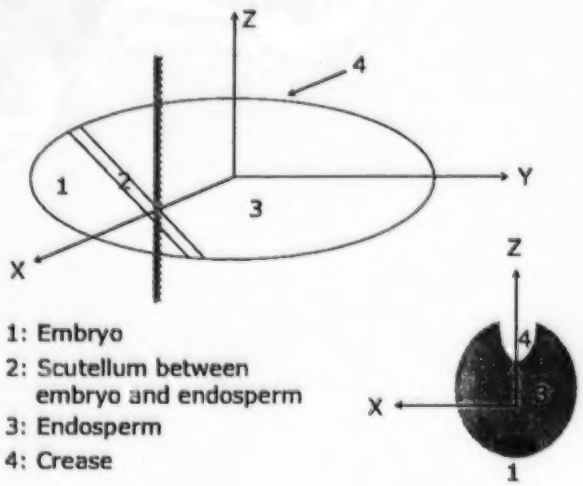


Figure 3. Very simple model of a barley kernel indicating the positions of the embryo and crease. An axis-system has been inserted to use as a guide for the interpretation of the MR images.

in the top diagram indicates the XZ-orientation used for some of the images. At the bottom of Figure 3 this orientation is shown as seen looking parallel to the Y-axis. Figure 4 shows 4 typical images of different kernels with at the top TR118 (Arborfield) images and at bottom left TR118 (Naicom) and at right Manley (St. Brieux) images, each used as a control. The left hand side corresponds to samples stored for 6 months while the right hand side represents samples stored for 7 months. The images show sections parallel to the long axis of the kernel and the embryo can be seen in all images. Hydration of the

endosperm takes more time, hence, the absence of signal intensity from this part of the seed. Images are displayed using a false colour scheme. Areas corresponding to a high water intensity are displays with red while the absence of water is marked by black. Signal intensity is scaled going from high to low as going from red, yellow, green, blue, purple to black. The TR118 Arborfield images show an increased water resonance intensity compared with the control samples at the bottom of the figure, especially in the area between the embryo and the endosperm. That the area between the embryo and endosperm could be damaged, either physically or chemically, is shown by some more images obtained from TR118 (Arbor-field) kernels, shown in Figure 5. This area is visible as a red band in the TR118 images, separating the embryo from the endosperm. This is not observed in viable control samples.

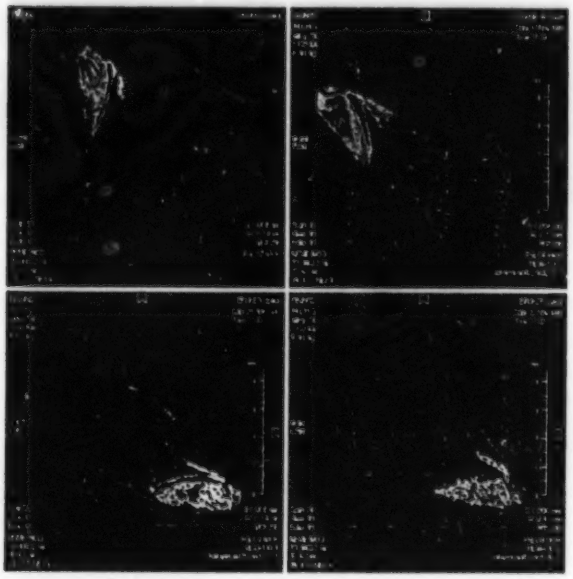


Figure 4. *MR Images of non viable TR118 (Arborfield) (top, left and right, after 6 and 7 months of storage, res-pectively) and TR118 (Naicom) (bottom, left, after 6 months of storage) and Manley (St. Brieux) (bottom, right, after 7 months of storage) as viable control. The TR118 images clearly show an increased water resonance intensity in the embryo/scutellum areas compared with the TR118 Naicom (bottom, left) and Manley St. Brieux (bottom, right). Areas corresponding to a high water intensity are displays with red while the absence of water is marked by black, using a scale going from high to low as going from red, yellow, green, blue, purple to black. Note that the images shown are typical for the ZY-plane orientation as explained in Figure 3.*

Corresponding MR-images for a TR118 (Arborfield) sample after 8 months storage are shown in Figures 6 and 7. Each Figure shows two perpendicular images of the same seed. The TR118 MR-images shown are representative of non-germinating kernels as germination, checked for after imaging, did not occur. These images show that for the TR118 (Arborfield) sample, hydration of the seed embryo occurs rapidly in comparison with images of the control sample, shown in Figure 4 (bottom) and Figure 8. In Figure 8, images of a control sample, Manley (St. Brieux) after 7 months storage, are shown. The bottom images, clearly show the growth of the roots from the embryo. Note that the image on the right hand side displays another orientation of the kernel, perpendicular to the image on the left. Figures 6 and 7 strongly suggest a degradation of the area between the embryo and endosperm in the TR118 (Arborfield) samples. In a few of the kernels studied, non-typical hydration patterns were observed which also indicate damage to

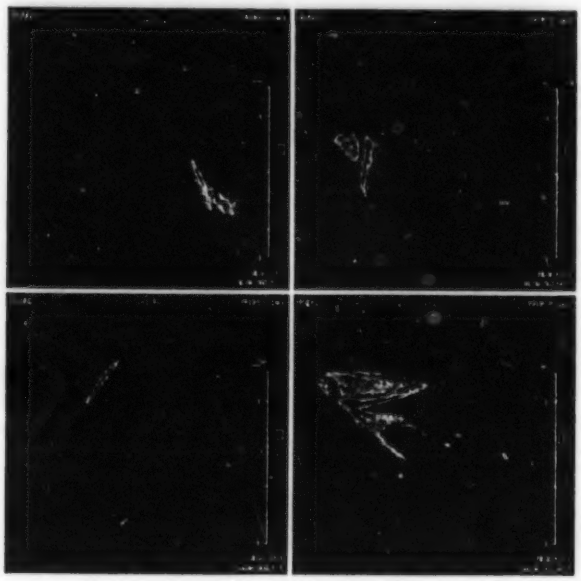


Figure 5. *MRI images of the ZY-orientation of a non viable TR118 (Arborfield) sample after 7 months of storage. The two top images show two different kernels, obtained after 6 hours after the initiation of the germination protocol. The two bottom images, also of different kernels, were obtained after 25 hours into the germination protocol.*

this area. Figure 9 shows a TR118 (Arborfield) kernel imaged 35 hours into the germination protocol. Hydration of the endosperm, from multiple points along the scutellum, indicates severe damage to the cell layer between the endosperm and embryo. A similar event occurs in Figure 6 at the left hand bottom.

Low seed viability (51%) was also observed for the Manley (Tisdale) sample after 8 months of storage (Table 1). Figure 10 shows typical MRI images of kernel water distributions of this sample. As observed for the TR118 (Arborfield) samples, these images show that hydration of the seed embryo occurs rapidly in comparison with images of viable control samples, especially in the scutellum area between embryo and endosperm.

MRI experiments indicate that samples with a known, reduced viability seem to be preferentially hydrated in the scutellum area prior to full hydration of the embryo. This was never observed for any of the control samples. Our observation of a fast hydration of the scutellum can in principle be explained by three basic scenarios:

- 1. Membrane damage in some parts of the embryo due to radicals (peroxides etc.).
- 2. A germination event prior to our experiments. During this attempt small molecules were produced after which hydration and germination were halted, followed by drying of the seeds.
- 3. During imbibition of barley kernels, the embryos pick up water very quickly. Therefore, under moist conditions, conducive to pre-germination, barley embryos would become hydrated and swell. When the kernels are subsequently dried, the embryos shrink and become relatively loose within the embryo cavity of the kernel. During re-hydration, water could rush into the cavity as well as being absorbed by the embryo.

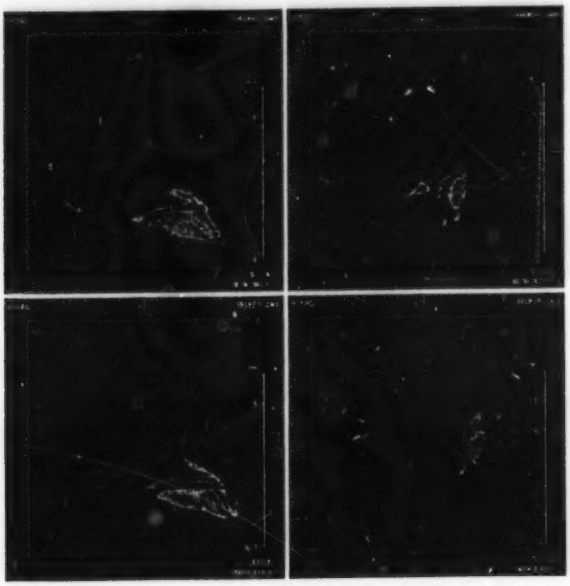


Figure 6. *MRI images of a non-viable TR118 (Arborfield) sample after 8 months of storage. The two top images show two orientations of the same kernel (ZY-orientation on the left and XZ-orientation on the right), obtained after 7 hours after the initiation of the germination protocol. The two bottom images, also of the same kernel (ZY-orientation on the left and XY-orientation on the right), were obtained after 10 hours into the germination protocol.*

The first scenario can be excluded as it does not explain the large α-amylase content of the seeds. This leaves us with scenarios 2 and 3 as likely explanations.

Scenario 3 requires a physical separation of the embryo from the rest of the kernel. If this occurs, a void between the endosperm and embryo would be created, which, in principle, could be filled with water upon hydration. To exclude this possibility, 8-months storage samples of TR118 from both Arborfield and Naicom were subjected to hyperpolarized ^{129}Xe NMR studies at the NRC Steacie Institute for Molecular Sciences (Drs. J. Ripmeester and I.L. Moudrakovski). These experiments did not show any significant penetration of Xenon gas into the kernels of the TR118 Arborfield sample. Additional experiments were performed with ^{19}F-Hexafluoroethane and kernels punctured with hypodermic needles prior to exposure to gas. No penetration of gas into the kernels could be observed, indicating that the voids are not likely to occur in these samples.

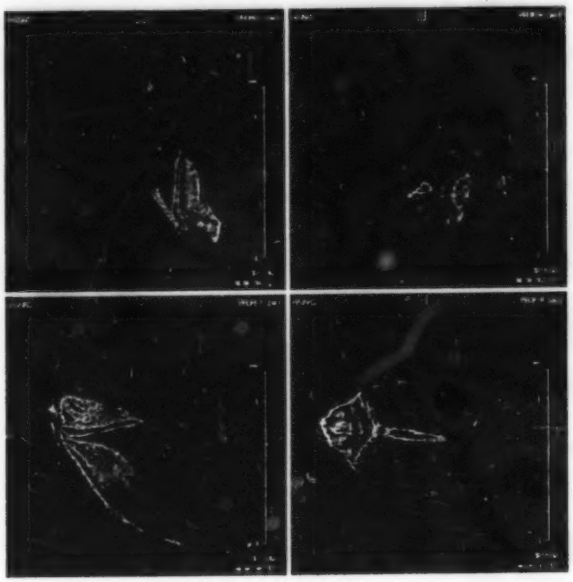


Figure 7. *MRI images of a non-viable TR118 (Arborfield) sample after 8 months of storage. The orientations are the same as in Figure 5A. The two top images show two orientations of the same kernel, obtained after 17 hours after the initiation of the germination protocol. The two bottom images, also of the same kernel, were obtained after 33 hours into the germination protocol.*

Assuming a previous hydration or germination attempt of the TR118 (Arborfield) and Manley (Tisdale) samples, sugars, proteins and lipids in the scutellum could have partially degraded to simple sugars, amino acids and fatty acids, which were left behind when this first germination at-

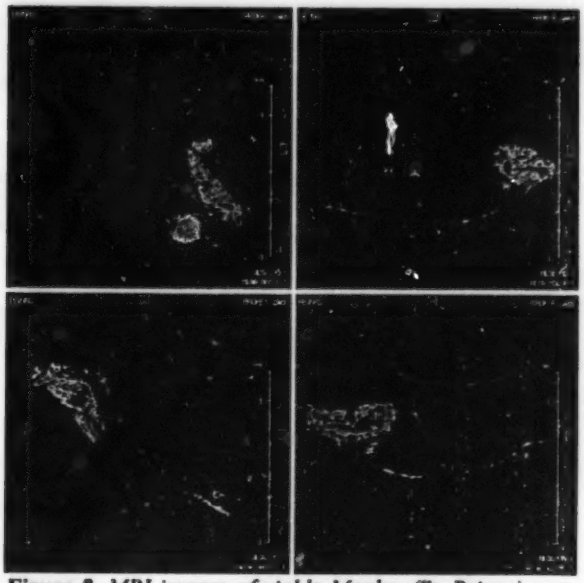


***Figure 8**. MRI images of viable Manley (St. Brieux) samples after 7 months of storage. The two top images show two orientations of the same kernel, obtained 13 hours after the initiation of the germination protocol. The two bottom images, also of the same kernel, were obtained after 21 hours into the germination protocol. All images represent ZY-orientations, except the right top image which shows an XY-orientation.*

tempt was halted when the seeds dried again. Upon a second germination attempt these sugars and amino acids created an osmotic potential, rapidly attracting water compared to other compartments of the embryo.

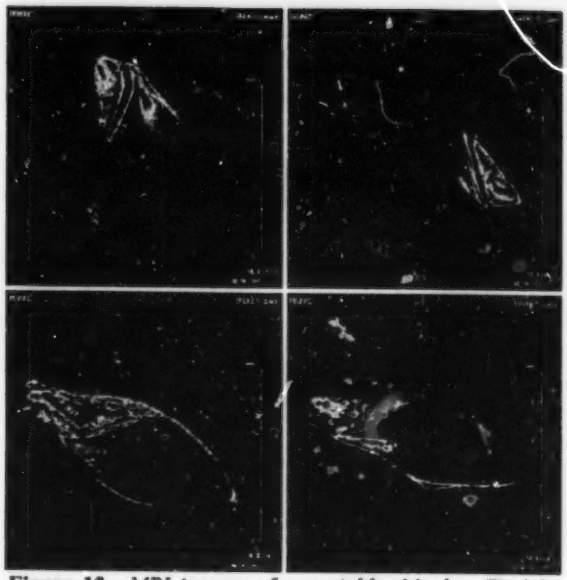


***Figure 10**. MRI images of non viable, Manley (Tisdale) after 8 months of storage, obtained, from left to right, 40 and 21 hours into the germination protocol. The two bottom images represent viable, Manley (St. Brieux) after 8 months of storage and were obtained after 38 and 19 hours, going from left to right respectively. All images show ZY-orientations.*

If this model is correct, profound hydration of the scutellum should result in a different overall water relaxation rate, as measured by NMR, compared to values obtained from sound samples. Water mobility in kernels can be measured with NMR spectroscopy, and can be used to characterize the physico-chemical state of water in biological materials using the ^1H water spin-lattice relaxation time (T_1) or rate ($R_1 = 1/T_1$). T_1 is very sensitive to water mobility and thus allows the indirect study of the water environment in kernels.

During imbibition, different parts of the kernels become hydrated at different times. This reflects itself in a continuous change in T_1 with time. We used imaging techniques which allowed the determination of water mobility in terms of T_1 and T_2. Table 5 summarize these relaxation studies and shows the presence of different water populations in the kernel, as characterized by their relaxation time constants.

← ***Figure 9**. An ZY-orientation MRI image of a TR118 (Arborfield) kernel after 7 months of storage . This image was obtained after 35 hours into the germination protocol and clearly shows the unconventional hydration pattern at the scutellum.*

	Scutellum	Endosperm	Embryo
T_1	450-650 ms	300 ms	350 ms
T_2	20±2 ms	15±3 ms	16±2 ms

Table 5. *MRI relaxation study results (n=3) of the TR118 (Arborfield) after 7 months storage and 39-41 hours of germination.*

Our relaxation studies indicate that the relaxation times (T_1 and T_2) observed for the scutellum are longer than those obtained for areas with similar high hydration levels. These observations fit our germination scenario well. Likely due to the presence of small molecular weight components (sugars and amino acids), water in this area of the seeds appears to be more mobile.

3.4. NMR Relaxation studies of single kernels.

Imbibition of barley is characterized by a rapid increase in ^{1}H R_1 during the first 6-7 hours of imbibition, followed by a small but steady decrease after reaching a maximum. The initial rapid increase in R_1 can be explained in terms of water uptake in porous materials. This process can be described by a simple diffusive pathway and predicts that the number of absorbed water molecules is proportional to the square root of the imbibition time (see below). In other words, the number of water molecules diffused into the barley kernels (N_{ABS}) during the imbibition time t, is proportional to the water distribution at time t. The width of the distribution is described by the water translational diffusion constant D.

$$N_{ABS}(t) = Const\sqrt{Dt}$$

During imbibition two populations of water exist, a so called "bound" water fraction and a fraction representing more mobile water molecules. From experiments in similar materials it is known that water molecules in these two states exchange rapidly compared to the T_1 of each of the sites. Dry barley still contains about 10% (by weight) water. This water occupies sites for bound water, indicated by the index *lm*. Sites for mobile water are indexed with *m*. Accordingly, the observed relaxation rate R_1 can be described by:

$$R_1^{Obs} = p_m R_1^m + (1 - p_m) R_1^{lm}$$

With the help of equation for $N_{ABS}(t)$, this can be written as:

$$R_1^{Obs} = R_1^{lm} + \frac{\sqrt{t}}{\sqrt{t} + \Xi}\left(R_1^m - R_1^{lm}\right)$$

Figure 11 shows a fit of R_1 *versus* the first 6 hours of imbibition of viable TR118 (Naicom) barley after 8 months of storage. Each data point represents an average measurement of R_1 performed on at least 4 different kernels. The kernels were not checked for germinative energy after the NMR measurement, however, it was known that the TR118 (Arborfield) sample had a very low germinative energy (20%, see Table 1). Figure 11 clearly shows that the water uptake in the TR118 (Arborfield) sample is different from that of the TR118 (Naicom) control sample after the

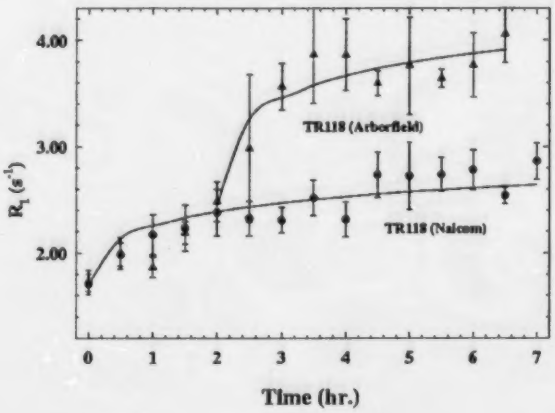


Figure 11. *^{1}H relaxation rates of TR118 (Arborfield) (Δ) and TR118 (Naicom) (o) barley, both after 8 months of storage, with a germinative energy of 20% and 99%, respectively. After approximately 3 hours of imbibition, the observed relaxation rate for TR118 (Arborfield) has become significantly different from that of the TR118 (Naicom) control. Data from the Naicom sample was fitted to R_1(Obs) while the curve for the Arborfield sample was drawn as a guide to the eye.*

first 2-3 hours. During the first 2-3 hours of imbibition both TR118 samples display similar relaxation rates. After approximately 2-3 hours of imbibition, the TR118 (Arborfield) sample shows a rapid increase in relaxation rate. The relaxation rates measured after 3 hours of imbibition for TR118 (Arborfield) are significantly different from those measured for the control and represent a second hydration phase. This observation could be explained introducing an

additional, new, porous area within the seed which has come into contact with water. This area could likely be the layer between the embryo and the endosperm. These relaxation results may form the basis of a simple test for barley viability.

A similar result was obtained for the Manley (Tisdale) sample after 8 months of storage. Compared to the control sample, Manley (St. Brieux), a general increase in the observed relaxation rates as a function of imbibition time was found. The data is shown in Figure 12. The viable Manley (St. Brieux) sample shows a smaller standard deviation of the observed rates than the Tisdale sample (51% viability according to Table 1). As kernels could not be pre-selected for the imbibition experiments, the larger error in the relaxation rate measurements found for the Manley (Tisdale) sample is related to nearly 50/50 distribution of viable and non-viable kernels in this sample, thus averaging relaxation data for viable and non-viable kernels.

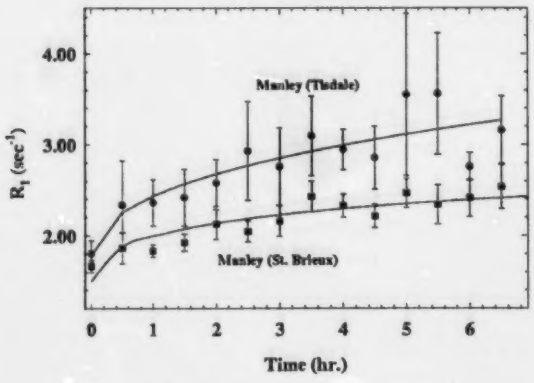


Figure 12. ^1H relaxation rates of Manley (Tisdale) (o) and Manley (St. Brieux) ( ) barley, both after 8 months of storage, with a germinative energy of 51% and 98%, respectively. Both curves for the Tisdale and St. Brieux samples were drawn as a guide to the eye.

These results suggest that the loss of viability during storage was caused by low levels of sprouting either before or after harvesting.

Using NMR relaxation measurements, the type of seed degradation found for the TR118 (Arborfield) and Manley (Tisdale) samples can easily be identified. For the TR118 samples, a significant difference in relaxation rates within 3-4 hours after imbibition, can be observed between viable and non-viable samples. The Manley samples, representing a 50/50 distribution of viable and non-viable kernels, showed a difference in relaxation rate after approximately 1 hour of imbibition.

3.5. Effect of storage conditions on seed viability.

Barley was stored in burlap bags in an area with poor ventilation, limited temperature control and mouldy walls. It is clear that the burlap bags do not offer any protection from this hostile environment. During storage in Nanjing, temperature and relative humidity were continuously recorded on charts (Table 6). The charts showed that temperatures fluctuated between 20 to 30°C while the relative humidity ranged from 60 to 90%. These conditions are far from ideal for the storage of malting barley.

α-Amylase levels in the control samples are shown in Table 2. Three samples contained elevated levels of α-amylase prior to shipment, Harrington (Carrot River), Manley (Tisdale) and TR118 (Arborfield), suggesting that these samples may have been subjected to low levels of pre-germination. Again, it should be noted that α-amylase levels in commercial malt are approximately 145,000 IDC units·g^{-1}. The highest α-amylase level recorded in Table 2 represents less than 0.5% of this value, indicating that the degree of pre-germination in these samples is exceedingly low. It appears to be sufficient, however, to damage the samples so that they lose viability when stored under unfavourable conditions of heat and high humidity (Table 6). Undamaged samples, those exhibiting low levels of α-amylase, maintain their viability under the same conditions.

Date (1999)	Temperature range (°C)	Relative Humidity range (%)
April 12-15	10-20	50-90
May 3-31	20-20	60-80
June 1-30	~25	70-90
July 1-18	~25	70-90

***Table 6.** Storage conditions of barley samples shipped to Nanjing, China.*

3.6. Meetings and seminars.

On October 30th, 1998, a one-day meeting was held at PBI and the University of Saskatchewan, Saskatoon, to develop a draft action plan for the study of barley germination loss during over-seas transport. Contributing participant were Marco L.H. Gruwel and Suzanne R. Abrams of PBI, Xiang Yin of PML., Alex W. MacGregor of the CGC Research laboratory, Michael Brophy of the CWB and Bryan Harvey and Eric Lefol of the UofS.

A second meeting of all participants was organized for October 21st, 1999, at the CGC Research Laboratory in Winnipeg, MB. All participants were able to attend and presented an update of their research and discussed future research plans.

During the PRRCG and Barley meetings, held in Saskatoon during the last week of February, 2001, researchers of PBI and PML organized a symposium on "Loss in germination capacity of malting barley during storage and handling", on February 27th. This symposium was very well attended and open to the public. All the institutes involved in the research project had one or more speakers present the latest research results. The feedback received by the organizers was very positive.

3.7. Conclusions and recommendations.

Using NMR Imaging we were able to show that for the barley samples with reduced viability a water concentration occurs in the scutellum area between the embryo and the endosperm. Imaging experiments clearly showed us the physico-chemical background related to dysfunctional seed physiology. This information could not have been obtained with any other experimental technique presently available. The exact origin of the seed germination dysfunction remains unknown, however it can likely be related to a pre-harvest germination attempt. With imaging experiments we were able to detect this unconventional water uptake after approximately 6-8 hours of germination.

Using the knowledge obtained from our imaging data; enhanced water uptake in the scutellum area between embryo and endosperm, we developed a simple NMR relaxation technique to discriminate between samples showing regular water uptake and samples with rapid uptake. These non-viable and viable samples show a significant difference in their water spin-lattice relaxation time, T_1. This difference can be rapidly detected after a few hours of imbibition of the kernels.

Discrimination of viable and non-viable samples based on T_1 has the advantage that it can be developed into a rapid screening technique. Relaxation measurements do not have to be performed on an instrument equipped with a high field superconducting magnet as used in our experiments. Small, mobile, NMR machines, equipped with electromagnets, are commercially available for these experiments.

We thus recommend further research into the application of NMR for the development of a seed viability test. At this point it should be mentioned that seed viability changes van have many different origins. Further research should resolve the question whether viability changes, independent of their origin, result in the same detectable physiological changes as observed in this study. Micro imaging experiments could certainly be optimized for acquisition time which will be accompanied by a reduction in resolution. This would allow imaging determinations after approximately 2 hours into germination, providing a rapid assessment of the water uptake pattern and physiological state of the kernel.

5. Acknowledgements

Support from the Agricultural Development Fund (ADF) has been acknowledged during tours of visitors to our NMR/MRI facilities. In addition ADF funding has been acknowledged on a recent presentation at the 3rd International Conference on "molecular biology and physiology of water and solute transport", July 2000, Goeteborg, Sweden, and at the February 27th, 2001, "loss in germination capacity of malting barley during storage and handling" symposium in Saskatoon, organized by PBI and PML.

6. Project Developed Materials

Publications

1. M.L.H. Gruwel, B. Chatson, X.S. Yin and S. Abrams, A magnetic resonance study of water uptake in whole barley kernels. Int. J. Food Sci. & Technol. 36, 161 (2001).
2. M.L.H. Gruwel, B. Chatson, X. Yin, S. Abrams, A nuclear magnetic relaxation study of water uptake in barley kernels in "Molecular Biology and Physiology of Water and Solute Transport", pp. 289, S. Hohman and S. Nielsen *Eds*. Kluwer Academic Publishers, New York, N.Y., 2000.
3. M.L.H. Gruwel, X.S. Yin, M. J. Edney, S.W. Schroeder, A.W. MacGregor, S.R. Abrams, barley viability during storage: the use of Magnetic Resonance as a potential tool to study viability loss. Submitted (2001).

Presentations

1. Cargill Worldwide Technology Meeting, Minneapolis, MN, USA, February 2000, X.S. Yin, G.S. Laycock, W.F. Fernet, S.R. Abrams, M.L.H. Gruwel, B. Chatson, E. Kendall, NMR microimaging of barley hydration and effect on metabolism.

2. Molecular Biology and Physiology of Water and Solute Transport, Goeteborg, Sweden, July 2000. M.L.H. Gruwel, B. Chatson, X. Yin, S. Abrams, A nuclear magnetic relaxation study of water uptake in barley kernels.

3. Symposium on Loss in germination capacity of malting barley during storage and handling, Saskatoon, SK, Canada, February 2001. M.L.H. Gruwel, Problems with germination of barley: what can we do with Magnetic Resonance ?